

Supplemental Material

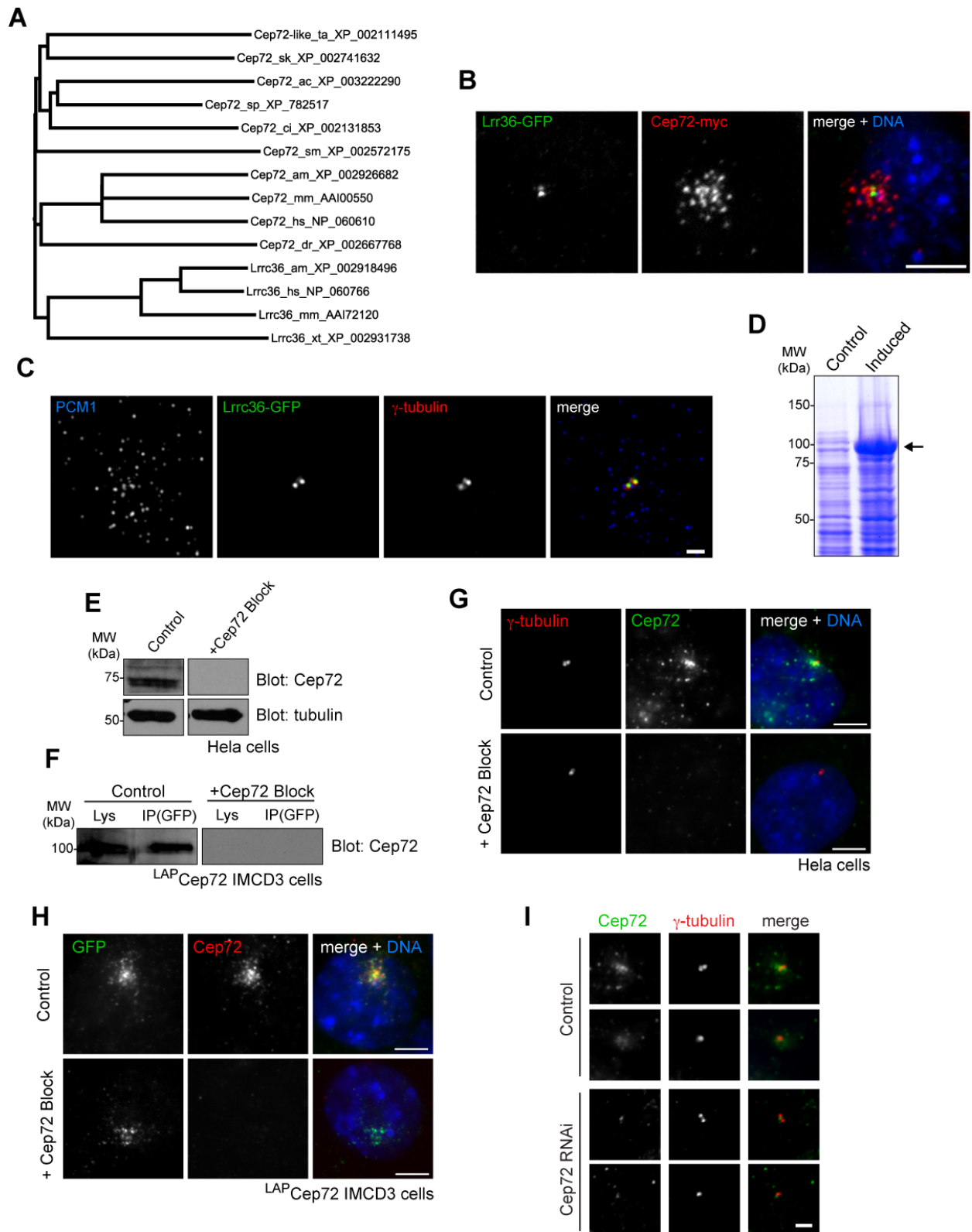


Figure S1. Phylogenetic analysis of Cep72 and Lrrc36, comparative localization of Cep72 and Lrrc36 and Cep72 antibody characterization (A) Phylogenetic alignment of Cep72 and Lrrc36 ortholog protein sequences. Vertebrate Cep72 orthologs are more related to individual Cep72-like orthologs in nonvertebrate organisms as compared to Lrrc36. (abbreviations – ta: *T. adhaerens*; ac: *A. carolinensis*; sp: *S. purpuratus*; ci: *C. intestinalis*; sk: *S. kowalevskii*; am: *A. melanoleuca*; mm: *M. musculus*; hs: *H. sapiens*; dr: *D. rerio*; sm: *S. mansoni*; xt: *X. tropicalis*). (B) To compare localization of Cep72 and Lrrc36, NIH3T3 cells were cotransfected with tagged cDNA expression constructs for each, fixed and immunostained for Cep72-myc (anti-myc), Lrrc36-GFP (anti-GFP) and DNA (DAPI). (C) hTERT-RPE1 cells were transfected with Lrrc36-GFP, fixed and immunostained for γ -tubulin, PCM1 and Lrrc36-GFP (anti-GFP). Note that Lrrc36-GFP localizes exclusively to the centrosome and does not overlap with PCM1. Images are deconvolved maximum projections of stacks from approximately 2 μ m sections. Scale bars, 1 μ m. Characterization of Cep72 antibody (D) Inclusion body preparation from control or GST-Cep72 expressing *E. coli*. Protein was transferred to nitrocellulose and the membrane region containing GST-Cep72 (~100 kDa) was excised and used for blocking experiments. (E) Western blot analysis of endogenous Cep72. Hela cell extracts were probed with Cep72 antibody, or γ -tubulin antibody as a loading control. Cep72 antibody detected a band of the predicted molecular weight for Cep72. This band was not detected when antibody was blocked with GST-Cep72. (F) Western blot analysis of ^{LAP}Cep72 using Cep72 antibody. ^{LAP}Cep72 was immunoprecipitated from ^{LAP}Cep72-IMCD3 cell extracts using an anti-GFP antibody. Input lysates (Lys) and immunoprecipitations (IP (GFP)) were probed with Cep72 antibody. Cep72 polyclonal antibody detected a band of the predicted molecular weight for ^{LAP}Cep72 in both extracts and immunoprecipitates. These bands were not detected when antibody was blocked with GST-Cep72. (G) Immunofluorescence analysis of endogenous Cep72. Hela cells were fixed and immunostained for Cep72 and γ -tubulin. Cep72 antibody labeled Cep72-positive particles surrounding the centrosome. Cep72 signal was absent when cells were stained with antibody blocked with GST-Cep72. (H) Immunofluorescence analysis of ^{LAP}Cep72. ^{LAP}Cep72-IMCD3 cells were fixed and stained for ^{LAP}Cep72 with Cep72 antibody and GFP antibody. ^{LAP}Cep72 was not detected when cells were stained with Cep72 antibody blocked with GST-Cep72, whereas ^{LAP}Cep72 was still detected with GFP antibody. DNA was stained with DAPI. Scale bars, 5 μ m. (I) Specificity of Cep72 antibody demonstrated by Cep72 RNAi. hTERT-RPE1 cells were transfected with control or Cep72-targeting siRNAs for 48 hours after which time cells were fixed and stained for endogenous Cep72 and γ -tubulin

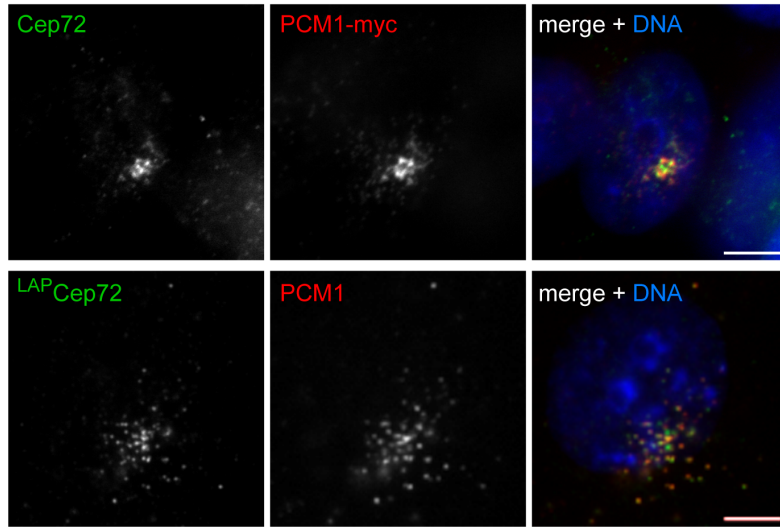
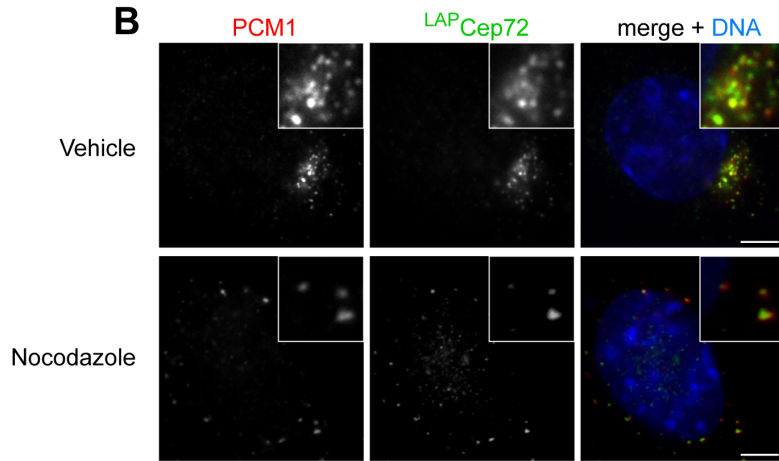
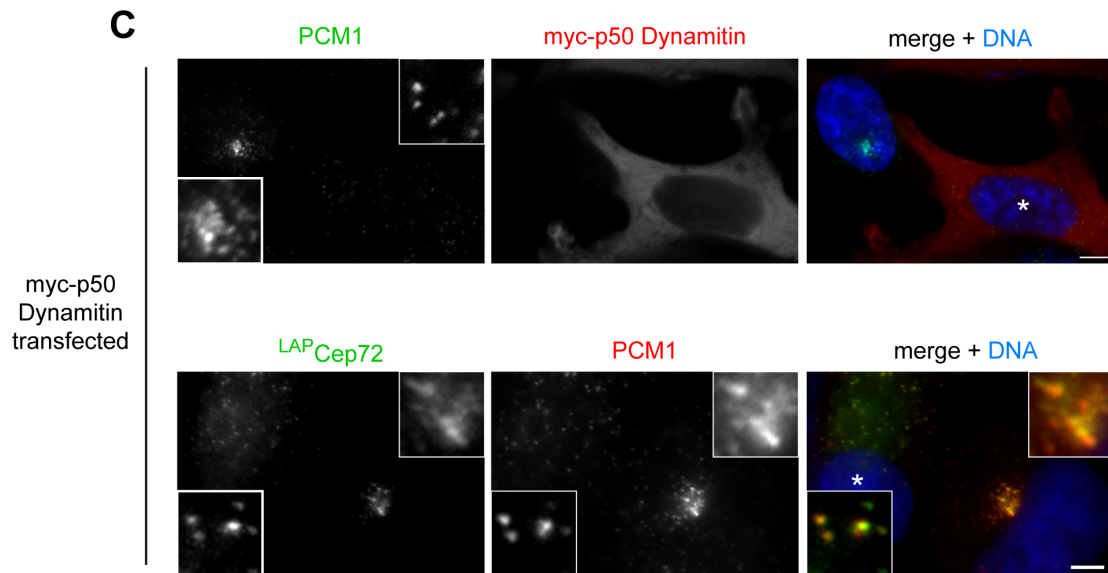
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Figure S2. Cep72 colocalizes with PCM1 and maintains this colocalization in nocodazole-treated and p-50-dynamitin-expressing cells. (A) HeLa cells were transfected with PCM1-myc, fixed and immunostained for Cep72 and PCM1-myc (anti-myc). DNA was stained with DAPI (top panel). ^{LAP}Cep72-IMCD3 cells were fixed and immunostained for ^{LAP}Cep72 (anti-GFP) and PCM1. DNA was stained with DAPI (lower panel). (B) Effects of microtubule depolymerization on distribution of ^{LAP}Cep72. ^{LAP}Cep72-IMCD3 cells were treated with DMSO (vehicle, top panel) or nocodazole (5 µg/ml, lower panel) for 3 h, fixed and immunostained for ^{LAP}Cep72 (anti-GFP) and PCM1. DNA was stained with DAPI. In control cells, ^{LAP}Cep72 colocalized with PCM1 in centriolar satellites. In nocodazole-treated cells, ^{LAP}Cep72 remained colocalized with PCM1, but was dispersed throughout the cytoplasm. (C) Effects of dynein disruption on distribution of ^{LAP}Cep72. ^{LAP}Cep72-IMCD3 were transfected with p50-dynamitin-myc, fixed and immunostained for p50-dynamitin-myc (anti-myc) and PCM1. DNA was stained with DAPI. PCM1 signal is dispersed throughout the cytoplasm in the p50-dynamitin-myc expressing cell (labeled with *, top panel). Transfected ^{LAP}Cep72-IMCD3 cells were fixed and immunostained for ^{LAP}Cep72 (anti-GFP) and PCM1. DNA was stained with DAPI. Both ^{LAP}Cep72 and PCM1 are dispersed throughout the cytoplasm, but remain colocalized in the p50-dynamitin-myc expressing cell (labeled with *, bottom panel).

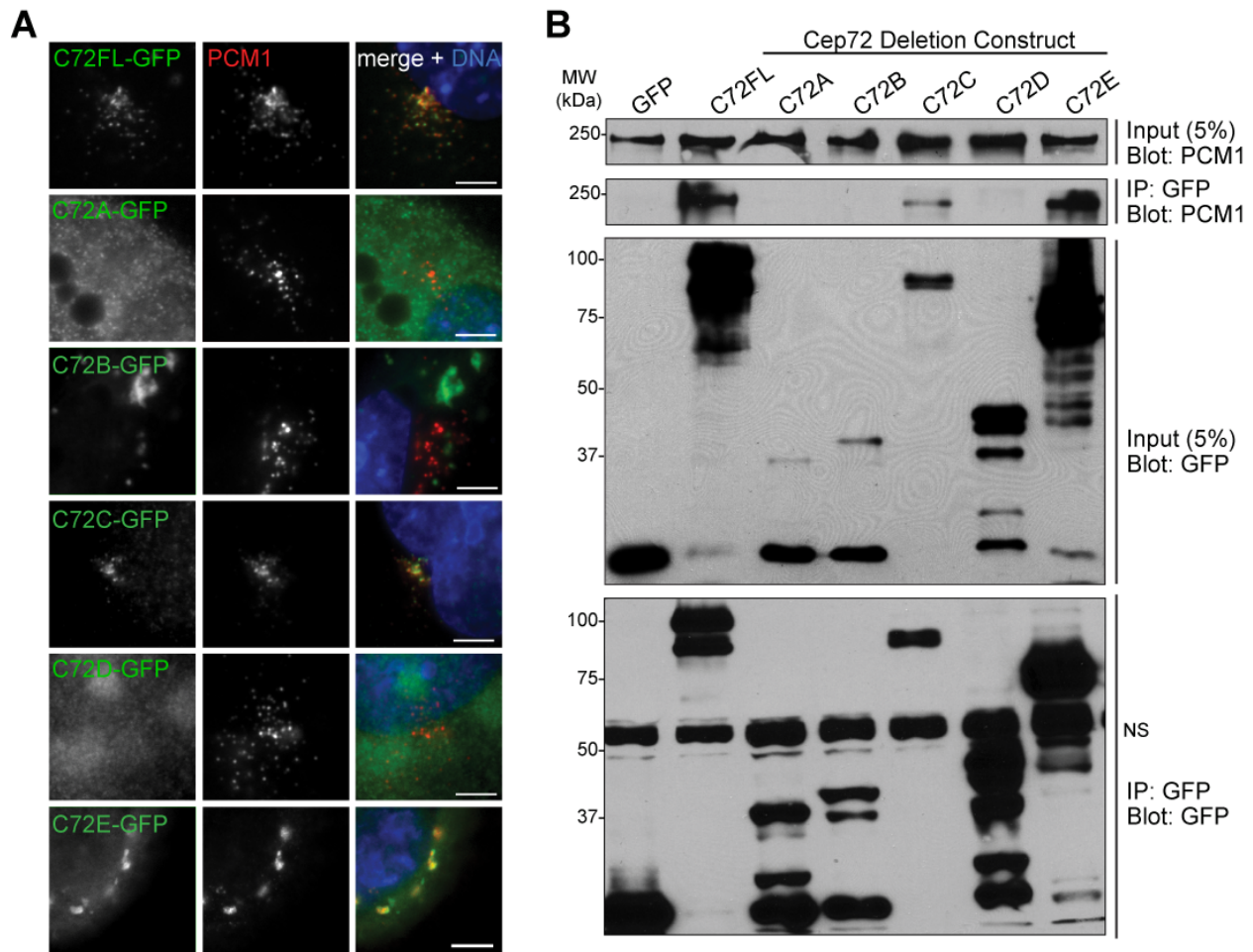


Figure S3. . Cep72 deletion analysis of PCM1 interaction. (A) Localization of Cep72 deletion constructs. NIH3T3 cells were transfected with GFP or GFP-tagged Cep72 deletion constructs, fixed and immunostained for GFP (anti-GFP) and PCM1. DNA was stained with DAPI. Scale bars, 5 μ m. (B) Immunoprecipitation of Cep72 deletion constructs and PCM1. NIH3T3 cells were transfected with GFP or GFP-tagged Cep72 deletion constructs. Extracts from transfected cells were probed with GFP antibody. Major bands detected in each case correspond to a fusion protein of the predicted molecular weight. (B) Interaction of Cep72 deletion constructs with PCM1. Immunoprecipitation of GFP or GFP-tagged Cep72 deletion constructs was performed on extracts from transfected NIH3T3 cells. Major bands detected in anti-GFP probed blots correspond to a Cep72 deletion GFP-fusion proteins of the predicted molecular weight. Inputs and immunoprecipitates were analyzed by western blot, probing for endogenous PCM1. NS, nonspecific band.

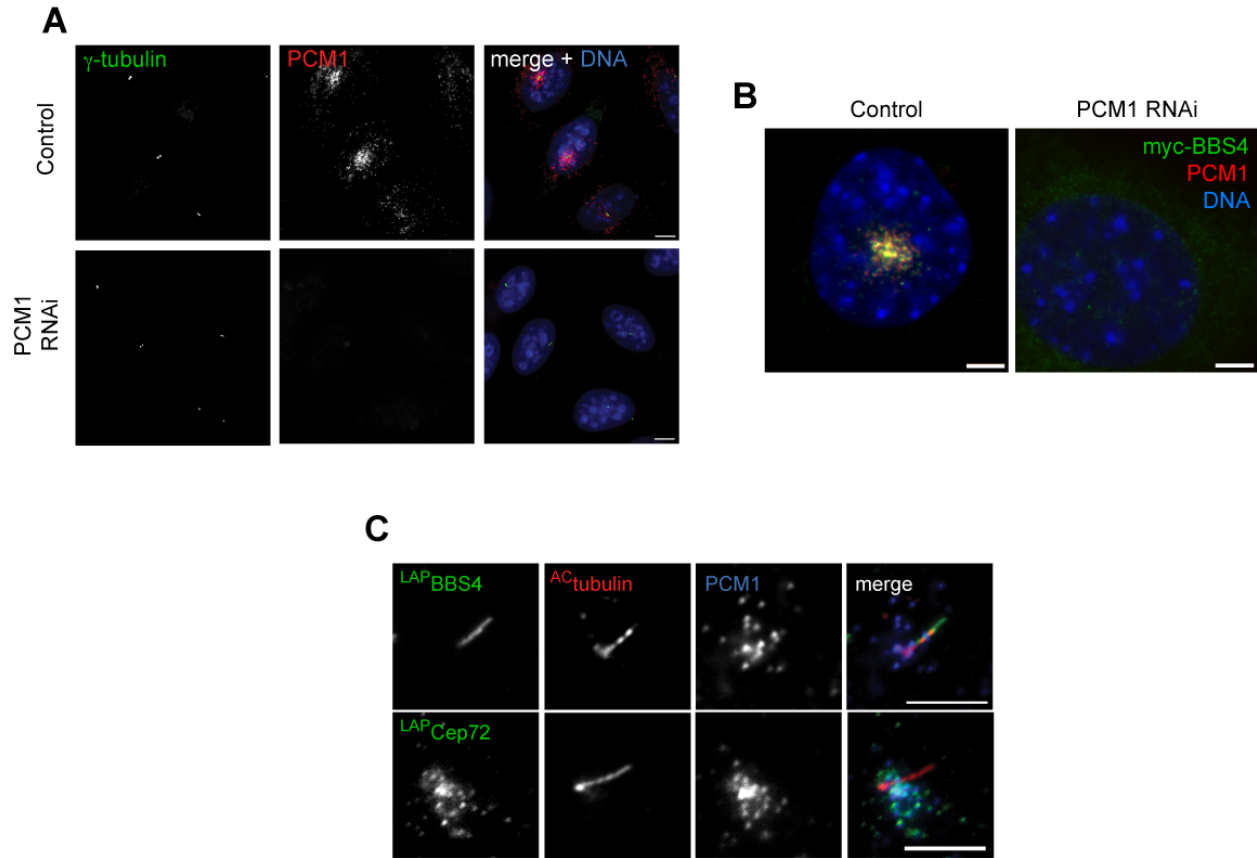


Figure S4. (A) Immunofluorescence analysis of PCM1-depleted cells. IMCD3 cells were infected with control lentivirus expressing a puromycin resistance gene or lentivirus expressing shRNA targeting PCM1 and a puromycin resistance gene. Cells were infected overnight, selected for 48 h in puromycin, allowed to recover for 24 h and fixed for immunofluorescence. Cells were immunostained for PCM1 and γ -tubulin. DNA was stained with DAPI. Scale bars, 5 μ m. (B) Control or PCM1 RNAi cells were transfected with myc-BBS4, fixed and immunostained for BBS4-myc (anti-myc) and PCM1. DNA was stained with DAPI. Scale bars, 5 μ m. (C) Localization of BBS4 and Cep72 in ciliated cells. Stably expressing ^{LAP}BBS4-hTERT-RPE1 cells or ^{LAP}Cep72-IMCD3 cells were serum-starved for 24 h to induce formation of primary cilia. Cells were fixed and immunostained for ^{LAP}BBS4 (anti-GFP, top panel), ^{LAP}Cep72 (anti-GFP, bottom panel), ^{AC}tubulin (to label primary cilia) and PCM1. Note that in ciliated cells, ^{LAP}BBS4 localizes to the primary cilium (top panel), whereas ^{LAP}Cep72 remains colocalized with PCM1 in the cytoplasm. Scale bars, 5 μ m.

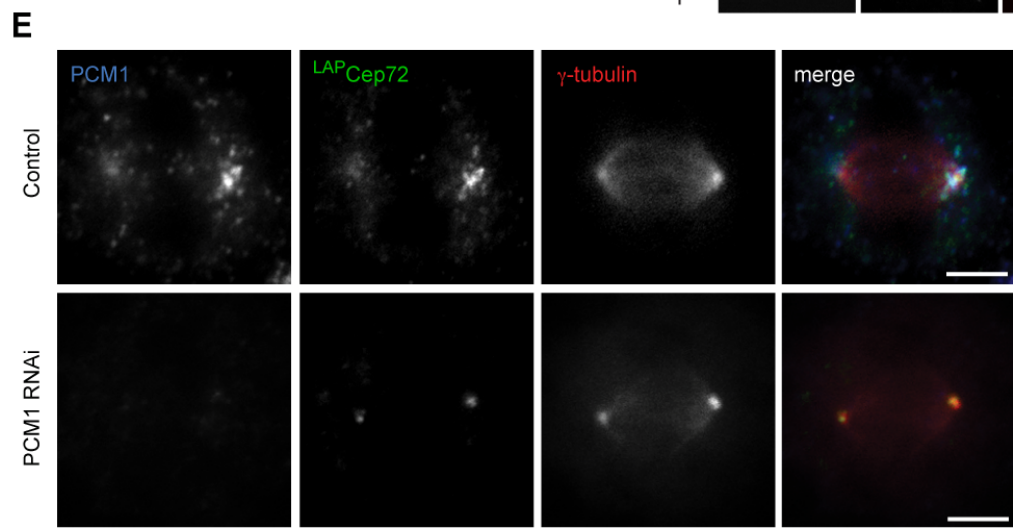
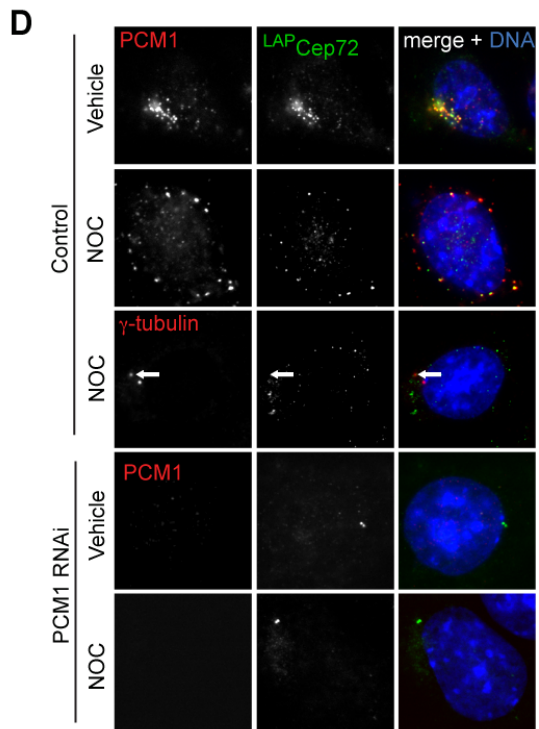
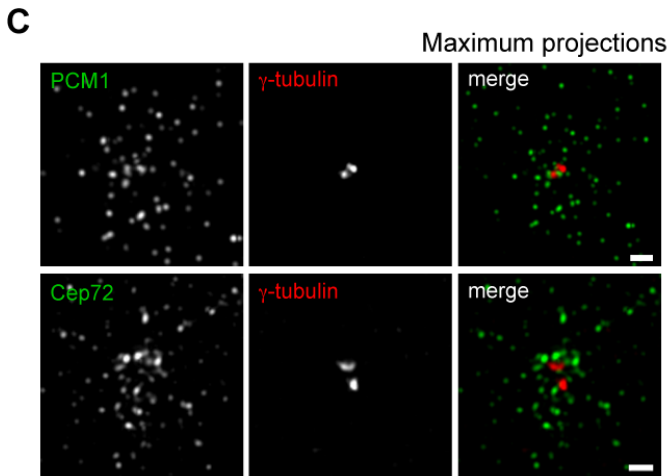
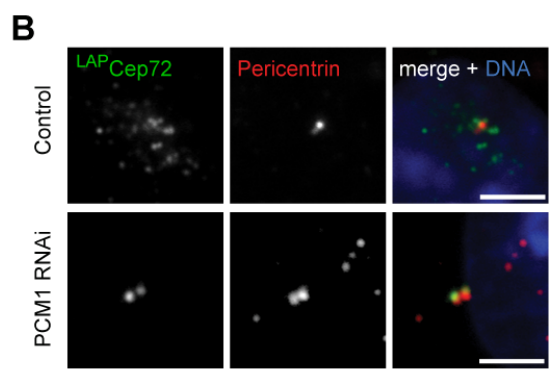
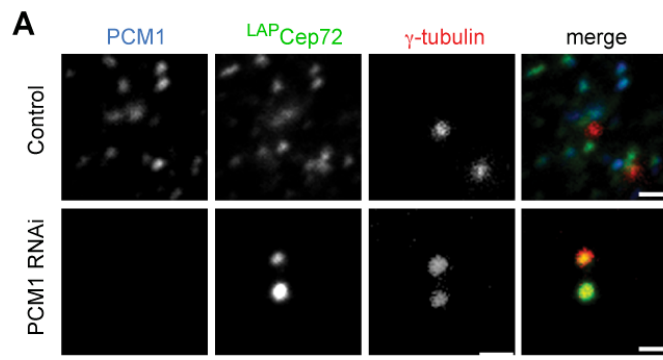


Figure S5. Effects of PCM1 depletion on centrosomal redistribution of Cep72. (A) Control or PCM1-depleted ^{LAP}Cep72-IMCD3 cells were fixed and immunostained for ^{LAP}Cep72 (anti-GFP), PCM1 and γ -tubulin or (B) ^{LAP}Cep72 (anti-GFP), pericentrin and DAPI (DNA). Colocalization of ^{LAP}Cep72 and centrosomal markers, γ -tubulin and pericentrin was observed only in the absence of PCM1. Scale bars, (A) 1 μ m, (B), 5 μ m. (C.) Deconvolution analysis of centriolar satellite distribution around the centrosome. Cells were fixed and immunostained for γ -tubulin and endogenous PCM1 (top) or endogenous Cep72 (bottom). Images represent maximum projections of stacks from approximately 2 μ m-thick sections. Scale bars, 1 μ m. (D) Control or PCM1-depleted ^{LAP}Cep72-IMCD3 cells were treated with vehicle or nocodazole for 4 hours. PCM1 and ^{LAP}Cep72 remain colocalized in nocodazole-treated cells, but are no longer focused around or localized to the centrosome and are dispersed through the cytoplasm. In vehicle and nocodazole-treated PCM1 depleted cells, ^{LAP}Cep72 remains localized to the centrosome indicating that microtubules are not required to maintain this localization. Arrow indicate centrosome in nocodazole-treated cells. Scale bars, 5 μ m (E) Mitotic control or PCM1-depleted ^{LAP}Cep72-IMCD3 were fixed and immunostained for ^{LAP}Cep72 (anti-GFP), PCM1 and γ -tubulin. In contrast to control cells, PCM1-depletion resulted in colocalization of ^{LAP}Cep72 and γ -tubulin at spindle poles. Scale bars, 5 μ m. (D, upper)

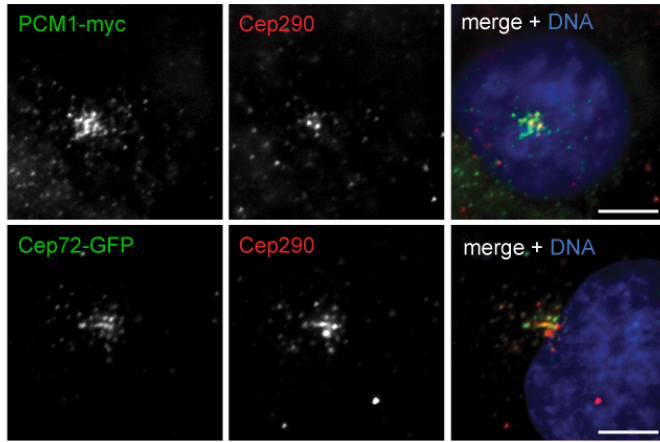
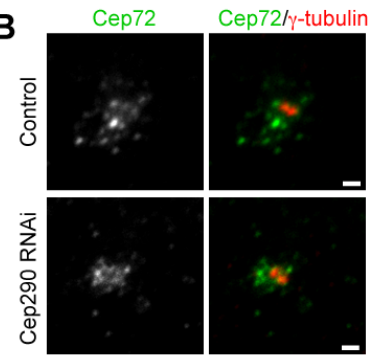
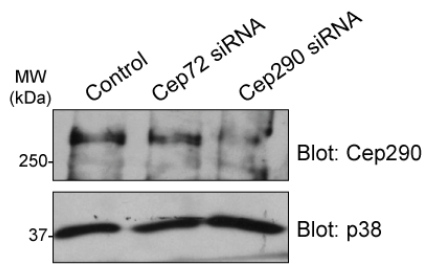
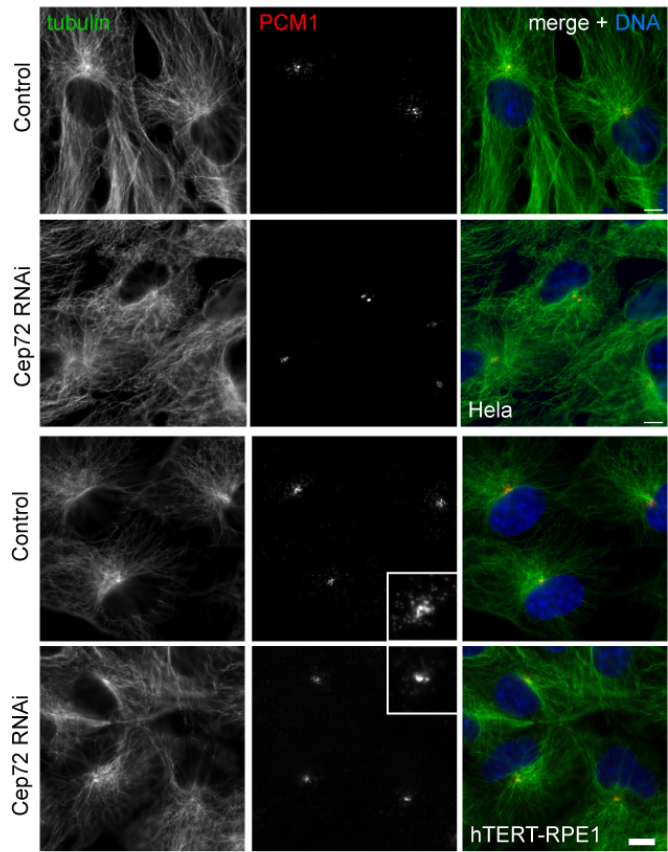
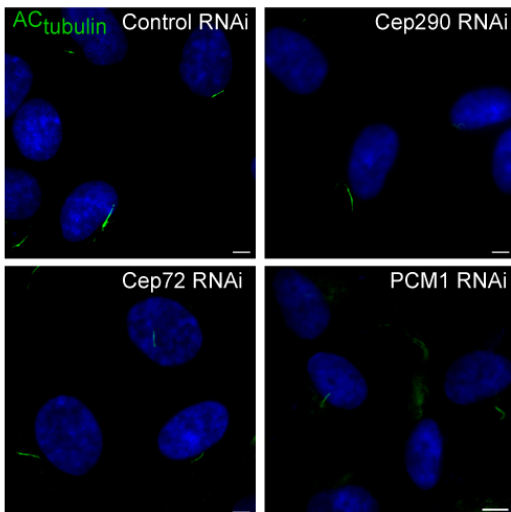
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Figure S6. Effects of Cep72 or Cep290 depletion. (A) Assessment of colocalization of PCM1-myc and Cep290. HeLa cells were transfected with PCM1-myc, fixed and immunostained for PCM1-myc (anti-myc) and Cep290. DNA was stained with DAPI. Scale bars, 5 μ m. (A, lower) Assessment of colocalization of Cep72-GFP and Cep290. hTERT-RPE1 cells were transfected with Cep72-GFP, fixed and immunostained for Cep72-GFP (anti-GFP) and Cep290. DNA was stained with DAPI. Scale bars, 5 μ m. (B) Cep290 is not required for localization of Cep72 to centriolar satellites. Control or Cep290 RNAi HeLa cells were fixed and immunostained for Cep72 and γ -tubulin. Cep290 was not required for recruitment of Cep72 to centriolar satellites, however Cep290 depletion resulted in a slight accumulation of Cep72-containing centriolar satellites around the centrosome. Scale bars, 5 μ m. (C) Cep72 depletion does not alter Cep290 protein levels. Extracts from hTERT-RPE1 cells transfected with control, Cep72 or Cep290 siRNA were prepared and probed for Cep290 and p38, as a loading control. (D) Organization of interphase microtubules is not disrupted in Cep72-depleted cells. HeLa cells (top) or hTERT-RPE1 cells (bottom) were transfected with control siRNA or Cep72 siRNA for 48 h. Cells were fixed and immunostained for PCM1 and tubulin. DNA was stained with DAPI. Cep72 depletion caused accumulation of PCM1 around centrosomes, but did not affect the organization of interphase microtubule arrays. Scale bars, 5 μ m. (E) Effects of Cep72, Cep290, or PCM1 depletion on primary cilium formation. hTERT-RPE1 cells were transfected with control siRNA, Cep72 siRNA, Cep290 siRNAs or a plasmid expressing a PCM1-targeting shRNA for 48 h, serum-starved for 24 h to induce cilium formation and fixed for immunofluorescence. Cells were immunostained for acetylated-tubulin to label primary cilia. DNA was stained with DAPI. Scale bars, 5 μ m.

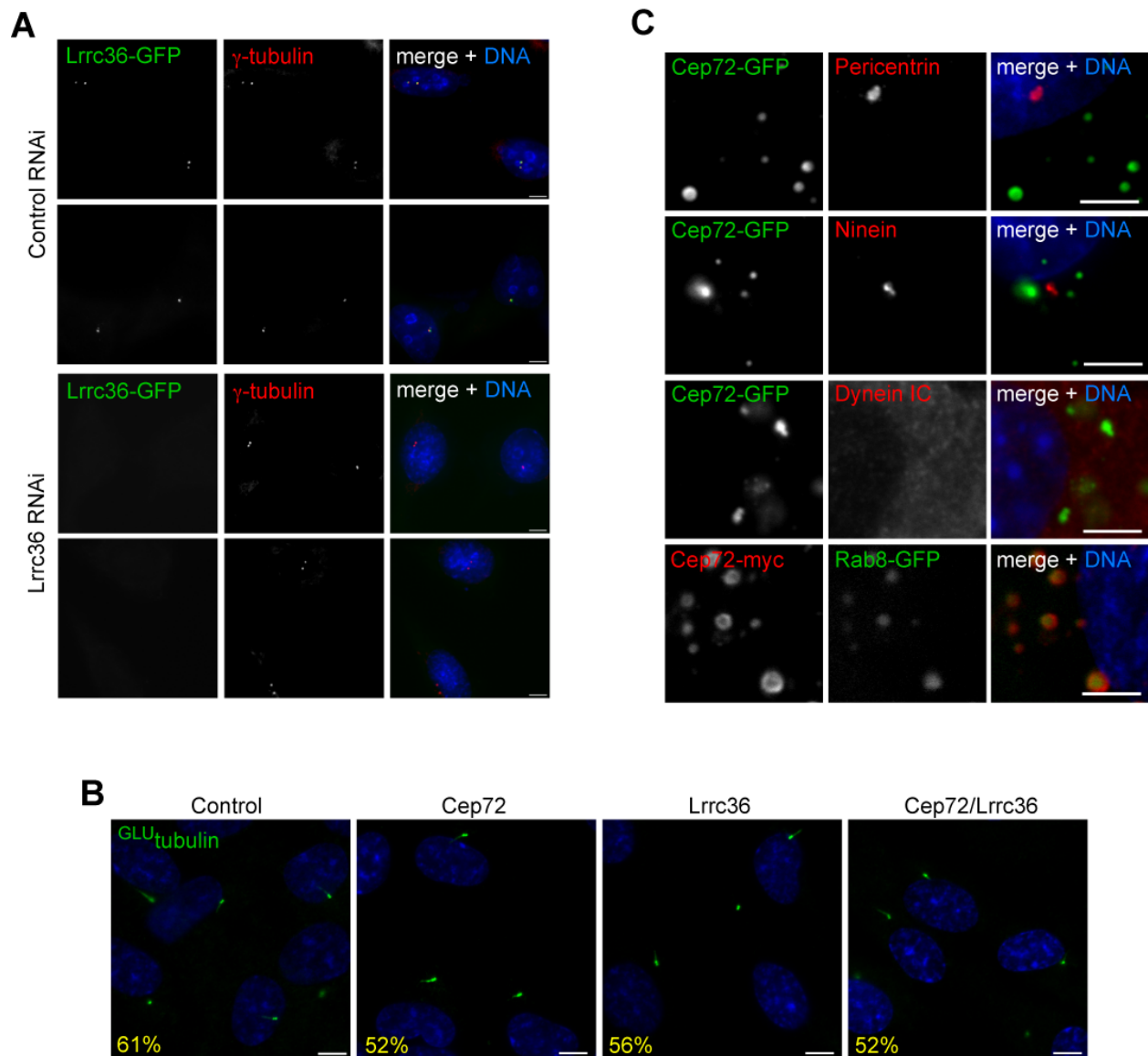


Figure S7. (A) RNAi-mediated depletion of Lrrc36. hTERT-RPE1 cells stably expressing Lrrc36-GFP were transfected with control siRNA or an siRNA pool targeting Lrrc36 for 48 h. Cells were fixed and immunostained for Lrrc36-GFP (anti-GFP) and γ -tubulin. DNA was stained with DAPI. Lrrc36-GFP signal was absent in cells transfected with the Lrrc36 siRNA pool (top and bottom panels are separate representative images from each siRNA transfection). (B) Codepletion of Cep72 and Lrrc36 does not affect cilium formation. hTERT-RPE1 cells were transfected with control siRNA, Cep72 siRNA, Lrrc36 siRNAs or both Cep72 and Lrrc36 siRNAs for 48 h, serum starved for 24 h and fixed for immunofluorescence. Cells were immunostained for glutamylated tubulin to label primary cilia. DNA was stained with DAPI. Depletion of Cep72, Lrrc36 or both did not affect primary cilium formation. Percentage of ciliated cells was determined for each case (lower left, n=100). Scale bars, 5 μ m. (C) hTERT-RPE1 cells were transfected with Cep72-GFP, fixed and immunostained for Cep72-GFP (anti-GFP) and either ninein, pericentrin or dynein intermediate chain (IC) (top three panels). Stably expressing Rab8-GFP-hTERT-RPE1 cells were transfected with Cep72-myc, fixed and

immunostained for Cep72-myc (anti-myc) and Rab8-GFP (anti-GFP) (bottom panel). DNA was stained with DAPI. In each case, high Cep72-expressing cells that contained Cep72 aggregates were examined. Scale bars, 5 μm .

Movie S1 3-Dimensional distribution of centriolar satellites in control siRNA-transfected cells. hTERT-RPE1 cells were transfected with control siRNA for 48 h, fixed and immunostained for PCM1 and γ -tubulin. Movies represent 360° forward and reverse rotations of 2 μm -thick sections centered on the centrosome. Scale bars, 5 μm .

Movie S2 3-Dimensional distribution of centriolar satellites in Cep72 siRNA-transfected cells. hTERT-RPE1 cells were transfected with Cep72 siRNA for 48 h, fixed and immunostained for PCM1 and γ -tubulin. Movies represent 360° forward and reverse rotations of 2 μm -thick sections centered on the centrosome. In Cep72 RNAi cells, PCM1 clusters tightly around the centrosome, but does not colocalize with γ -tubulin. Scale bars, 5 μm